

**SURFACE AREA MAPPING AND RINSE PROCEDURES OF RAW PRODUCE
TO DETERMINE EFFECTIVENESS OF PATHOGEN REMOVAL**

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

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September 6, 2002

Blacksburg, Virginia

Keywords: *Salmonella*, Ultrasound, Surface Area, Apples, Strawberries, Cantaloupe

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ABSTRACT

Bacterial pathogens on the surfaces of raw produce may be difficult to remove for identification and enumeration. The first part of this project examined whether ultrasonic treatment (40 kHz) of a rinse solution would enhance recovery of *Salmonella* spp. from various produce surfaces. Strawberries, apples, and cantaloupe were surface inoculated with a five-strain cocktail of nalidixic acid resistant *Salmonella* spp. Samples were subjected to one of six different treatments using different combinations of agitation methods (manual shaking or ultrasound), diluent temperatures (25°C and 40°C), and agitation times (60 and 120 seconds). After treatment, diluent was spiral plated onto tryptic soy agar supplemented with 50 ppm of nalidixic acid and plates were incubated at 37°C for 48 hours. Results from this study indicate that ultrasonic treatment of a rinse solution did not enhance or diminish recovery of *Salmonella* spp. from produce surfaces, as compared to manual agitation. The effects of diluent temperature and exposure time appeared to have a significant effect on recovery, depending on the type of produce.

The second part of this project used a computer imaging system to determine the surface area of various types of produce. The imaging system acquired and stored multiple images of the produce samples. From these images, surface fitting and approximation of a 3-D wire frame model were used to calculate surface area. From

these measurements, it was determined that there were statistical relationships between surface area and weight. Surface area measurements were used to develop equations to predict surface area from weight measurements.

ACKNOWLEDGEMENTS

I would like to thank Dr. Joseph Eifert for being my advisor and giving me guidance during the course of my research. I would also like to thank Dr. Susan Sumner and Dr. Merle Pierson for serving on my committee. For media preparation and help with data collection, I would like to thank Lindsay Millard, Robert Gunter, and Marcus Obusan. I would also like to thank my lab mates and friends, Renee Raiden, Brooke Seeman, Angela Hartman, Alison Roberts, Kali Phelps, Megan Hereford, Karol Gailunas, and Christine Piotrowski, for their help and for keeping me company in lab. For technical support, I would like to thank Brian Yaun, Brian Smith, and Dr. Hengjian Wang. For help with statistical analyses, I would like to thank Wes Schilling, from Virginia Tech, and Dr. Hans J. Carter, from Virginia Commonwealth University. For developing the surface area measurement software, I would like to thank D.J. Lee. A special thanks goes to David Rasmussen, for inspiring me to pursue my Masters degree. Last, but not least, I would like to thank all of my family and friends who have supported me during my six years here at Virginia Tech.

This research was partially funded through the Cooperative State Research, Education, and Extension Service of the U.S. Department of Agriculture, Special Research Grants Program – Food Safety (Project Number 99-34382-8463).

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LITERATURE REVIEW

A. Foodborne Illness Linked to Produce Consumption

1. Minimal Processing of Produce

Fresh fruits and vegetables are an important part of the diet of many people all over the world. Physicians and health officials recommend consuming more produce and fewer foods with saturated fat in order to promote a healthier diet. Because of this trend towards healthier eating, the consumption of fresh fruits and vegetables has increased. Since fresh fruits and vegetables undergo a minimal amount of processing, care must be taken at the preharvest, harvest, and postharvest level in order to ensure high quality products, and to minimize contamination by pathogenic microorganisms. Minimal processing of fruits and vegetables has two purposes: 1) to keep the produce fresh without losing nutritional quality and 2) to have a shelf life sufficient to make distribution feasible within a region of consumption (Ahvenainen, 2000).

Fruits and vegetables are harvested at various times of the year, depending on the type of product and season. The nature of some fruits and vegetables requires careful handpicking in order to reduce damage to the product, which can affect overall quality (Yildiz, 1994). Mechanical harvesting aids such as hydraulic platforms and ladders can aid workers in the field by lifting them to elevated heights in order to pick products from trees. However, care must be taken at harvest time to ensure products are picked at the right time. Harvesting at the correct time can be estimated by crop scheduling systems or heat unit systems. If produce is harvested at the wrong time, a decrease in product quality can occur. For example, studies have shown that grapes harvested when fruit temperature was high (above 30°C) had poor color and contained high levels of acetic acid and alcohol, indicating microbial contamination and spoilage (Yildiz, 1994).

At harvest time, other processing techniques and equipment can be utilized in the field. Products such as beans and peas can be shelled and threshed using combines. Dry sorting of fruits and vegetables removes damaged or grossly contaminated products. Fruits and vegetables can be washed in the field using a 0.1% non-alkaline wetting agent to remove insects, then a water spray can be applied to remove the wetting agent, debris, chemical residues, and insects (Yildiz, 1994). After harvesting and washing, fruits and vegetables can be packed into pallet bin boxes or shipping containers for precooling (Yildiz, 1994). Rapid precooling of the fruits and vegetables is important because it removes the field heat and the heat of respiration. Precooling can be accomplished by use of forced-air cooling, vacuum cooling, or hydro air cooling (Yildiz, 1994). It is important that any equipment or storage container, used in the field or the processing facility, be in clean condition in order to prevent spoilage or contamination by microorganisms (Yildiz, 1994).

Transportation of the produce, whether from the fields or to supermarkets, must be carefully done. Storage containers and refrigeration temperatures are important factors that must be monitored. Containers must protect the produce from mechanical damage that can occur during transport. For example, berry fruits should be packaged in shallow containers to prevent them from crushing under their own weight (Yildiz, 1994). Containers must be of good condition because if the container has burrs or rough edges, it may cause damage to the fruits or vegetables (Brackett, 1994). If the product, especially juicy or nutrient-laden products, is damaged, the juices from the product may leak onto other products, which may encourage microbial growth (Brackett, 1994). Maintaining refrigeration temperatures during transport or at any other point in the processing

operation is also important, especially for perishable products. Refrigeration systems usually consist of a compressor-condenser-evaporator unit that is located away from the storage unit. A fan is used to draw air from the evaporator and discharges it into a ceiling duct that is located above the storage unit (Yildiz, 1994). Mixtures of various gases, such as CO₂, N₂, O₂, etc. can be used to create a modified atmosphere (Yildiz, 1994). The mixture of gases is dependent on the type of produce being transported. Proper temperatures must be maintained in order to slow or inhibit microbial growth. Of particular concern are psychrotrophic microorganisms, which can survive refrigeration temperatures and can proliferate at ambient temperatures (Brackett, 1994). Many of the psychrotrophic microorganisms are important pathogens or spoilage microorganisms (Brackett, 1994).

After transport, produce may be either sold whole or sliced and packaged. At the processing plant, fruits and vegetables can be sorted based on weight, shape, color, etc. Separation of produce into size and weight quality groups can provide uniformity of the finished products for buying and selling (Yildiz, 1994). Oversized, undersized, and damaged produce is separated from the rest of the products. One method of separation is screening, where the products pass over a vibrating screen and those products that are of acceptable size remain on the screen, while those that are undersized fall through the screen (Yildiz, 1994). Other methods of separation can include belt and roller sorters, flatbeds, drums, rollers, or light reflectance and transmittance (Yildiz, 1994).

Cleaning, washing, and disinfection of the produce are the next step after separation. Cleaning and washing involves the removal of soil, insects, twigs, stones, and pesticide residues from the produce (Yildiz, 1994). In the process line, produce is

generally washed in an enclosed chamber where the produce is agitated in water, while limiting human contact with the products (Yildiz, 1994). Water, with 50-200 ppm of chlorine, is commonly used to wash produce. The use of chlorine in wash water has been known to reduce microbial populations in lettuce and tomatoes, but at concentrations of 50 µg/ml, chlorine was ineffective at reducing populations of *Listeria monocytogenes* (Beuchat, 1995). While chlorine cannot be used for complete produce disinfection, it can be used to reduce the likelihood of microbial contamination (Beuchat, 1995). Water quality, whether used for cleaning or during any other step of the processing operation, must be carefully monitored because contaminated water can introduce pathogens or other microorganisms into the product (Tauxe, 1997).

After washing and disinfection, produce may undergo further processing. Peeling of the fruit or vegetable may be done. Some products, such as apples, oranges, potatoes and carrots, require peeling before further processing. Peeling involves the removal of the outer layer of the fruit or vegetable either by hand, with lye or other chemical alkalis, by mechanical means, with steam or boiling water, by flame, by freezing, etc. (Yildiz, 1994). Mechanical, chemical, or steam peeling are faster methods of peeling, but these processes may not be gentle, and can disrupt cell walls of the produce which can enhance microbial growth or enzymatic browning (Ahvenainen, 2000). Care must be taken at the peeling step because the outer layer of fruits and vegetables act as a barrier to prevent microorganisms from entering the interior of the product (Tauxe, 1997). Once the outer layer is removed, and if it is contaminated, microbial growth can be rapid (Tauxe, 1997).

Another step may involve the cutting of the produce. Cutting is a size reduction operation where the product is cut or broken into smaller pieces to either improve taste,

digestibility, ease of handling, or to have effective heat transfer (Yildiz, 1994). A sharp, thin knife generally performs cutting, but other methods can be used such as a water knife, which utilizes a fine jet of high-pressure water (Yildiz, 1994). When a product is cut, cellular respiration is increased, plant tissue is softened, and chemical reactions can occur depending on the type of product (Yildiz, 1994). Therefore, after cutting, the product must be stored under refrigeration conditions to maintain product quality and to inhibit microbial growth (Yildiz, 1994). It has been known that cutting can introduce microorganisms into the edible portion of the product. Once introduced into the interior, microorganisms can rapidly proliferate under the right temperature. Some microorganisms on the outer surface of the produce that are not considered as spoilage microorganisms may become so if introduced into the interior of the product (Brackett, 1994). Also, when fruits or vegetables are sliced, juices from these products may leak onto processing machinery, which can be utilized by microorganisms (Brackett, 1994). In order to prevent microbial growth, cutting equipment and machinery must be properly cleaned and sanitized.

After cutting, produce can be mixed and assembled. This can be performed with combined items such as bagged salads or ready-to-eat products. Mixing is done to ensure that a homogeneous mixture is formed and maintained with as low an energy output as possible at the lowest overall cost. Several different types of machinery can be employed. Tumblers can be used to gently mix solid items. Ribbon mixers are used for products that do not flow readily or for fine pastes. Agitators, with paddles and baffles, are often used for slow mixing of products (Yildiz, 1994).

The final step for minimally processed fruits and vegetables is assembly and packaging. Various processing and aseptic techniques can be utilized at this final step of the operation. In the assembly and packaging room, workers must wear mouth masks, hair nets, gloves, and a specified dress in order to prevent contamination of the final product. The environment of the assembly and packaging room is also carefully maintained. The room may utilize filtered air under positive pressure, with temperature maintained at 10-12°C and humidity at 60-70% RH (Yildiz, 1994). Packaging of the final product is important in that it prevents dehydration and allows for consumers to view and inspect the product (Brackett, 1994). Materials used in packaging must be clean or sterile in order to prevent contamination of the product. Modified atmosphere packaging can be utilized to inhibit microbial growth and to increase shelf-life of the product (Brackett, 1994). The basic principle behind modified atmosphere packaging is to create an atmosphere containing about 2-5% CO₂, 2-5% O₂, and the rest nitrogen in order to create an optimal gas balance to minimize respiration of the product (Ahvenainen, 2000).

2. Sources of Contamination

Contamination of produce can either occur in the preharvest or postharvest operation. During preharvest, microbial contamination can come from soil, water, animal feces, or improperly treated manure. In soil, pathogens such as *Clostridium perfringens*, *C. botulinum*, *Listeria monocytogenes*, and *Bacillus cereus* are common inhabitants, so their occurrence on produce is not uncommon (Beuchat and Ryu, 1997). The closer the produce is to the ground, the higher the risk of contamination. Water is another potential source of contamination. Water sources on the farm that are untreated, or that are

contaminated by raw sewage or polluted runoff from upstream livestock operations, can introduce pathogens into the produce (FDA, 1998). Many pathogens may be present in water contaminated by sewage, but *L. monocytogenes* is the most commonly isolated pathogen in sewage, with counts higher than that of *Salmonella* spp. (Beuchat and Ryu, 1997). Finally, animal fecal material and improperly treated manure is another source of contamination. Animal and human fecal material is considered a significant source of human pathogens (FDA, 1998). These materials can contaminate the produce if animals shed their feces where the product is grown, poor hygiene on the part of the worker handling the product, or if improperly treated manure is used as fertilizer.

Postharvest contamination sources may include water, processing equipment, human handling, transport operations, and product abuse after purchase. Water may serve as a source of contamination if processing water, used for rinsing or cooling, is reused. If water is reused or improperly treated, microbial loads may increase in the water and may contaminate the product (FDA, 1998). Unsanitary processing equipment may introduce microorganisms from the environment or other food sources and may contaminate the product. Also, the physical act of cutting may introduce microorganisms on the exterior of the produce into the edible portion of the product. In terms of human handling, workers can contaminate the produce. If a worker does not practice proper hygiene, they can contaminate not only the product, but also processing water, other workers, and equipment (FDA, 1998). For the transportation operation, contamination may occur if the transportation and storage facilities are unsanitary. Potential sources of contamination during transportation can include cross contamination with other foods, non-food sources, and unsanitary surfaces during the loading, unloading, storage, and

transportation operations (FDA, 1998). Also, if improper storage temperatures are maintained, microorganisms may proliferate on the product. Finally, product abuse after purchase can include cross contamination with other foods, improper storage temperature, or handling by a contaminated worker/consumer.

3. Microorganisms Associated with Raw Produce

Fresh fruits and vegetables can harbor a wide variety of naturally occurring microorganisms. Some of these microorganisms may or may not be spoilage or pathogenic. Microbial populations on these products can range from 10^5 - 10^7 CFU/g (Francis et al, 1999). Depending on whether the product is a fruit or vegetable, these products can contain different species of microorganisms.

Fresh vegetables can support microbial growth due to their neutral pH and high water and nutrient content (Brackett, 1994). Of all the microorganisms found on these products, about 80-90% of the population are gram-negative rods, such as *Pseudomonas*, *Enterobacter*, or *Erwinia* species (Francis, et al, 1999). Gram-positive bacteria are also commonly found, such as *Bacillus* or coryneform bacteria (Brackett, 1994). Lactic acid bacteria have also been found on products such as mixed salads and grated carrots (Francis et al, 1999). Yeasts and molds may also be found. Commonly occurring yeasts include *Cryptococcus*, *Candida*, and *Rhodotorula*, (Francis et al, 1999). Mold populations can include *Aureobasium*, *Fusarium*, *Alternaria*, *Epicoccum*, *Mucor*, *Chaetomium*, *Rhizopus*, and *Phoma* (Brackett, 1994). Microbial populations on vegetables can vary greatly. Vegetables can become contaminated with soil and sand, which can act as carriers of microorganisms. It is not uncommon to find microbial populations in the millions on fresh vegetables. Leafy vegetables can harbor a large

population of microorganisms, but vegetables grown underground, such as carrots and potatoes, can possess significantly lower microbial populations (Brackett, 1994). The population of microorganisms on vegetables depends on various growth, extrinsic, and environmental factors.

Fruits can harbor different microorganisms than that of vegetables. Fruits generally possess higher sugar content and more acidic pH, which inhibit bacterial growth (except that of lactic acid bacteria) and support the growth of various yeasts and molds (Brackett, 1994). Commonly occurring yeasts include *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Kloeckera*, *Candida*, and *Rhodotorula*. Molds can include *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Cladosporium*, and *Botrytis*. Populations of yeast and fungi can be quite high in fruits. Fungi populations can range from 38,000-680,000 fungi per gram (Brackett, 1994).

Spoilage bacteria are generally not pathogenic, but they can cause a significant decrease in product quality. A majority of spoilage caused by bacteria are usually gram-negative species (Brackett, 1994). One of the most common types of spoilage is called bacterial soft rot. Different bacteria can cause soft rot, but the most important are *Erwinia carotovora* and *Pseudomonas marginalis* (Jay, 2000b). Soft rot can occur on produce such as cantaloupe, watermelons, onions, tomatoes, beans, carrots, broccoli, garlic, lettuce, potatoes, cabbage, etc. The bacteria break down pectins with pectinases, which causes a soft, mushy consistency, bad odor, and a water-soaked appearance (Jay, 2000b). *P. marginalis* soft rot is very similar to *E. carotovora* soft rot, except that *P. marginalis* is a psychrotroph capable of rapidly growing at refrigeration temperatures (Brackett, 1994). Other types of bacteria can cause different spoilage conditions.

Potatoes may rot from a condition called “black leg”, caused by *E. carotovora* pv. *atroseptica* or *E. chrysanthemi*. *Xanthomonas* can form yellow mucoid colonies on products such as cabbage or mustard (Jay, 2000b).

Fungi are also capable of causing spoilage in fresh produce. *Botrytis cinerea* can cause a gray mold rot on strawberries, onions, garlic, asparagus, potatoes, cabbage, etc. This type of spoilage occurs in warm temperatures and high humidity, and is characterized by production of a gray mycelium on the fruit or vegetable. Sour rot, oospora rot, or watery soft rot is caused by *Geotrichum candidum*. *G. candidum* is transferred from the fruit fly to the produce and will only grow in the cracks or wounds of damaged fruits and vegetables. Another spoilage fungi transmitted by the fruit fly is *Rhizopus stolonifer*, which grows in cottony masses and makes produce soft and mushy (Jay, 2000b).

It has been known that some of the native microflora of fresh fruits and vegetables can be inhibitory to human pathogens. Liao and Fett (2001) examined the effect of culture media and incubation time on the recovery of native microflora from three types of produce (Romaine lettuce, prepeeled baby carrots, and green bell peppers) and two types of seeds (alfalfa and clover), determined the APC of each produce and identified special groups of microflora, and selected for strains that were antagonistic to human pathogens. There was no significant difference between counts on three different media: *Pseudomonas* agar F, brain heart infusion agar, and plate count agar (Liao and Fett, 2001). Incubation temperatures had a different effect on recovery. Growth was observed at all three incubation temperatures (8°C, 28°C, and 37°C), with 28°C producing the highest recovery (Liao and Fett, 2001). Large amounts of growth were also observed at

8°C, indicating that these microorganisms were capable of surviving refrigeration temperatures (Liao and Fett, 2001). On plate count agar, 23-73% of the bacteria isolated were fluorescent pseudomonads, which have been found to be a large proportion of the native microflora of vegetables (Liao and Fett, 2001). Pectolytic bacteria, which are believed to play a role in spoilage, accounted for 6% of the aerobic microflora in bell peppers, 13% in Romaine lettuce, and 18% in baby carrots (Liao and Fett, 2001). Forty-six strains of pectolytic bacteria were isolated and included species such as *Pseudomonas*, *Erwinia*, *Bacillus*, *Xanthomonas*, and *Flavobacterium* (Liao and Fett, 2001). Finally, approximately 120 isolated strains of native microflora were tested for their ability to inhibit growth of *Listeria monocytogenes*, *Salmonella* Chester, *Escherichia coli*, or *Erwinia carotovora* subsp. *carotovora*. Six of the strains, *Bacillus pumilus*, *B. mojavensis*, *B. megaterium*, *Pseudomonas aeruginosa*, *P. fluorescens*, and yeast, were found to suppress the growth of at least one pathogen (Liao and Fett, 2001). All strains, except *B. pumilus*, inhibited the growth of *L. monocytogenes*, *E. coli*, and *E. carotovora* subsp. *carotovora*. *B. pumilus* was only capable of inhibiting *L. monocytogenes* (Liao and Fett, 2001).

4. *Salmonella*

Salmonellae are rod-shaped, motile, enteric Gram-negative bacteria members of the group Enterobacteriaceae. There are over 2,700 serotypes of Salmonellae. It is estimated that *Salmonella* spp. causes approximately 1.4 million cases of illness each year in the United States. Of these cases, less than 500 are fatal and 2% of all cases result in chronic arthritis (CDC, 1999b). Two serotypes, Typhimurium and Enteritidis, have been associated with half of all cases of salmonellosis (CDC, 1999b).

Salmonella spp. are most often found in animals. Their primary habitat is the intestinal tract of birds, reptiles, farm animals, humans, and sometimes insects. In the intestines, Salmonellae are excreted through feces and may contaminate food or water (Jay, 2000a). Salmonellae can also be found in different regions of the body. In a study of slaughterhouse pigs, Salmonellae were found in lymph nodes, diaphragm, spleen, and liver, with the lymph nodes having a higher incidence of Salmonellae than feces (Kamelpacher, 1963).

According to Jay (2000a), Salmonellae can be divided into 3 groups. The first group is those serovars that only infect humans, such as Typhi, Paratyphi A, Paratyphi C, and all others that cause typhoid and paratyphoid fever. The second group consists of the host-adapted serovars, such as Gallinarum, Dublin, Abortus-equi, and Choleradis. In this group, some of these serovars are human pathogens and can be transmitted through food. The third group consists of unadapted serovars with no host preferences. This group is pathogenic for humans and animals, and includes most of the foodborne serovars.

Salmonella spp. can cause 3 different types of illness. The most common form of illness is enterocolitis. In the United States, enterocolitis can be caused by 1500-2000 serotypes (Brooks et al, 1998). Acute symptoms of enterocolitis, 8-48 hours after ingestion, can include nausea, vomiting, abdominal cramps, diarrhea, headache, and low-grade fever. The infective dose for enterocolitis is approximately 10^7 to 10^9 cells total, but in some cases, the infective dose can be as low as 100 cells. Symptoms generally last for 1 to 2 days, but may be prolonged depending on host factors, ingested dose, or strain characteristics (FDA, 2002c). The second type of illness that can occur is bacteremia

with focal lesions. *S. Choleraesuis* is most commonly associated with this type of illness, but it can be caused by any serotype (Brooks et al, 1998). After ingestion, the bacteria invade the bloodstream and may cause lesions in the lungs, bones, meninges, or other areas (Brooks, et al, 1998). The third type of illness is typhoid fever, mainly caused by *S. Typhi*. After ingestion, the bacteria enter the small intestine, pass through the lymphatic system, and then into the bloodstream. After a 10-14 day incubation period, symptoms can include fever, malaise, headache, constipation, bradycardia, myalgia, formation of rose spots on the skin, and intestinal hemorrhage and perforation (Brooks et al, 1998).

Salmonellae are most commonly found in foods such as eggs, poultry, meat, and meat products. Salmonellae can be found in other foods such as milk, mayonnaise, cake mixes, dough, coconut meal, salad dressing, etc. (Jay, 2000a). Although the number of illnesses associated with *Salmonella* spp. and fresh produce is low, the bacteria can survive on the outer surfaces of these products. If Salmonellae are introduced into the interior of the product, rapid growth and proliferation can occur.

Knudsen et al (2001) examined the survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on whole and cut strawberries stored at ambient, refrigerated, and frozen temperatures. Although there was an initial population reduction after inoculation and drying of the strawberries, it was found that both pathogens were able to survive on cut and intact strawberries at all three storage temperatures. Although there were minor reductions in bacterial numbers, populations remained fairly constant. Due to the low pH of the strawberries (3.2-4.1), both pathogens were able to survive, but not grow, on the surfaces and interior of the strawberries.

Golden et al (1993) examined the growth of five *Salmonella* spp. (Anatum, Chester, Havana, Poona, and Senftenburg) in the interior of cantaloupe, watermelon, and honeydew melons. The strains selected were responsible for salmonellosis outbreaks associated with watermelon and cantaloupe. It was found that *Salmonella* spp. can survive in the interior tissues of the fruit at 5°C, and can proliferate at 23°C.

Zhuang et al (1995) examined the growth and survival of *S. Montevideo* on the outer surface of tomatoes. After storage at 10°C for 18 days, the population of *S. Montevideo* did not change significantly (Zhuang et al, 1995). However, there were significant increases in population within 7 days when the tomatoes were stored at 20°C and 30°C.

5. Other Bacterial Pathogens of Concern

Fruits and vegetables can harbor a wide range of naturally occurring microorganisms that are indigenous to the environment in which they are grown. Most of these microorganisms are harmless, but some can be pathogenic. Bacterial pathogens other than *Salmonella* spp. can include *Clostridium botulinum*, *Shigella* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Aeromonas hydrophila*.

Of all the bacterial pathogens, *Clostridium botulinum* is the pathogen of greatest concern in ready to use vegetables (Jay, 2000b). *C. botulinum* is a gram-positive, anaerobic, spore-forming rod commonly found in soil and water. It is capable of growing at pH values greater than 4.6 and its spores are resistant to heat treatment. *C. botulinum* produces a neurotoxin that, 12 to 72 hours after ingestion, will cause flaccid paralysis of muscles, fatigue, dizziness, headache, respiratory failure, and death if not treated (Jay, 2000c). Although botulism is often associated with canned foods, it can occur in fresh

produce. For example, botulism has been associated with cabbage mixed with coleslaw dressing (pH = 3.5), where *C. botulinum* apparently grew on the cabbage and produced toxin, even when the cabbage was packaged under modified atmosphere conditions (Beuchat, 1995). In a study with romaine lettuce and cabbage, Petran et al. found that *C. botulinum* grew and produced toxin in vented packages of lettuce and in nonvented packages of cabbage held at 21°C for 21 and 7 days (Beuchat, 1995).

Shigella is another pathogen of concern in fresh produce. *Shigella* is a member of the Enterobacteriaceae family and is composed of four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (Jay, 2000a). This gram-negative bacterium causes shigellosis, or bacillary dysentery, characterized by diarrhea (sometimes bloody), fever, and abdominal pain (Brackett, 1994). The infective dose can be as low as 10 cells (Jay, 2000a). Shigellosis can be transmitted by contaminated water or foods, or through person-to-person contact (Beuchat, 1995). *Shigella* has been associated with outbreaks with produce that received little or no heat treatment. At a university in Texas, lettuce contaminated with *S. sonnei* caused students to become ill (Beuchat, 1995). In another outbreak associated with lettuce, a worker infected with *S. sonnei* contaminated lettuce at a produce facility, which was distributed to restaurants where customers became ill (Beuchat, 1995). In a study examining the growth and survival of *Shigella* on sliced fruit, *Shigella* grew on watermelon, jicama, and papaya cubes when stored at 25-27°C for 6 hours (Escartin et al, 1989). In another study, *S. sonnei* survived on lettuce stored at 5°C for 3 days without a decrease in numbers, with increased growth at 22°C (Beuchat, 1995).

Escherichia coli is another important foodborne pathogen. Five virulence types of *E. coli* are recognized: enteroaggregative (EaggEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) (Jay, 2000e). Of the five types, only two, ETEC and EHEC, are important to produce. Enterotoxigenic *E. coli* is a common cause of travelers' diarrhea, and is capable of causing illness in both children and adults (Jay, 2000e). The estimated infective dose of ETEC is in the range of 10^8 - 10^{10} CFU (Jay, 2000e). ETEC, associated with travelers' diarrhea, is often associated with contaminated vegetables (Beuchat, 1995). In one outbreak, 47 airline passengers became ill after consuming a salad containing iceberg and romaine lettuce, endive, and shredded carrots (Beuchat, 1995). Enterohemorrhagic *E. coli* is another important type associated with produce. Of the 30 different types of EHEC, *E. coli* O157:H7 is recognized as the prototype for this group (Jay, 2000e). *E. coli* O157:H7 is often associated with undercooked meat and dairy products, but can be found in fresh produce as well. Outbreaks have occurred where salad ingredients were cross contaminated with raw ground beef (Beuchat, 1995). Unlike other virulence groups, *E. coli* O157:H7 is capable of producing a potent toxin that damages the intestinal lining (FDA, 2002b). This toxin, known as Shiga toxin or verotoxin, is similar to toxins produced by *Shigella dysenteriae* (FDA, 2002b). The infective dose of *E. coli* O157:H7 is unknown, but as few as 10 cells is suspected to cause illness (FDA, 2002b). Symptoms usually involve severe abdominal cramping along with diarrhea that starts out as watery, but then becomes grossly bloody (FDA, 2002b). While the illness only lasts a few days, this pathogen is of particular concern to the young and elderly. Children may develop hemolytic uremic syndrome (HUS), which is characterized by renal failure and

hemolytic anemia, which may eventually lead to kidney failure (FDA, 2002b). The elderly may experience HUS, along with fever, neurologic symptoms, and thrombocytopenic purpura (TTP) (FDA, 2002b). Illness caused by *E. coli* O157:H7 has a mortality rate of up to 50% for the elderly (FDA, 2002b).

Listeria monocytogenes is also a pathogen of concern in fresh produce. It is a short Gram-positive rod that has tumbling end over end motility at 22°C. Its optimal growth temperature is 37°C, but it has the ability to survive at refrigeration temperatures. *L. monocytogenes* is widely distributed in nature, and has been isolated from vegetables such as asparagus, cucumbers, cabbage, lettuce, potatoes, and other unprocessed vegetables (Martinez et al, 2000). The infective dose for *L. monocytogenes* is unknown, however it is assumed that less than 1,000 cells can cause infection in certain individuals (FDA, 2002a). The bacteria do not affect healthy adults and children. *L. monocytogenes* is of major concern to people who are immunocompromised, elderly, or pregnant. About 12 or more hours after ingestion, acute symptoms may include fever, headache, nausea, vomiting, and diarrhea (FDA, 2002a). The more serious symptoms may appear a few days or weeks. This can include meningitis, encephalitis, septicemia, and intrauterine/cervical infections that may result in spontaneous abortion in pregnant women (FDA, 2002a). *L. monocytogenes* is associated with foods such as raw milk, pasteurized milk, ice cream, produce, raw and cooked meat, and cheeses. In a study examining growth rates of *L. monocytogenes* in ready to serve lettuce, growth rates were variable (Steinbrugge et al, 1988). In two trials at 5°C, *L. monocytogenes* grew to 2.9×10^6 and 4.2×10^5 CFU/g at 7 days, but no growth was detected at 14 days (Steinbruegge

et al, 1988). In another trial at 12°C, *L. monocytogenes* grew to 1.2×10^7 CFU/g after 7 days, but declined to undetectable levels at 14 days (Steinbruegge et al, 1988).

Another psychrotroph of concern with produce is *Aeromonas hydrophila*. *A. hydrophila* is an aquatic gram-negative, facultatively anaerobic bacteria commonly found in untreated water and animals associated with water, such as fish or shellfish (Martinez, 2000). *A. hydrophila* usually causes a mild diarrheal illness, but it is capable of producing a more severe illness, characterized by bloody or mucus-containing diarrhea (Brackett, 1994). Because it is indigenous to the environment, it is not uncommon to find *A. hydrophila* on fresh produce. Studies have found *A. hydrophila* were found on vegetables such as asparagus, broccoli, and cauliflower (Berrang et al, 1989). In one study, *A. hydrophila* grew equally well on asparagus, broccoli, and cauliflower, with populations starting below the detection level (10^2 cells/g) at initial storage and growing to over 10^6 cells/g after 21 days of storage at 4°C (Berrang et al, 1989).

6. Outbreaks Associated with Fresh Produce

Between 1973 and 1987, *Salmonella* spp. accounted for 42% of all foodborne disease outbreaks and 51% of cases due to foodborne bacterial pathogens (Bean and Griffin, 1990). During this time period, *Salmonella* spp., along with *Bacillus cereus*, *Campylobacter*, *C. botulinum*, *C. perfringens*, *Shigella*, and *Staphylococcus aureus* accounted for 93% of outbreaks and 94% of cases (Bean and Griffin, 1990). Most of the *Salmonella* related infections involved beef, poultry, and egg products (Bean and Griffin, 1990). Recently, the incidence of *Salmonella* illnesses has been on the decline. Between 1996-1998, there has been a 13% decline in the number of *Salmonella* infections and a 44% drop in the incidence of *Salmonella* Enteritidis (CDC, 1999a).

Even though *Salmonella* infections are going down, overall, a higher proportion is linked to raw produce. In 2002, the I. Kunik Company of McAllen, Texas, issued a voluntary recall of its Susie brand cantaloupe because they were contaminated with *Salmonella* Poona. The cantaloupe, which were imported from Mexico and distributed in the United States, were linked to outbreaks of salmonellosis in the United States and Canada (FDA, 2002d).

Other *Salmonella* outbreaks have been associated with cantaloupe. Cut cantaloupe is capable of fostering growth of pathogenic bacteria due to its low acidity (pH 5.2-6.7) and high water activity (A_w 0.92-0.97) (FDA, 2001). Between June and July 1991, more than 400 infections of *S. Poona* occurred in 23 states (CDC, 1991). The suspected cantaloupe were believed to have originated from Texas and most of the cases involved fruit salads or salad bars held at room temperature for several hours (CDC, 1991). Another outbreak involved *S. Chester* that resulted in 25,000 infections occurring in 30 states, and resulting in 2 deaths (Madden, 1992). Here, it was suspected that *S. Chester* on the unwashed rinds of the cantaloupe were introduced into the interior of the fruit, and then held and served at various salad bars (Madden, 1992).

Salmonella spp. is a problem on other melon-type fruits. In 1955, *S. Miami* and *S. Bareilly* caused multiple outbreaks associated with pre-cut watermelons (Beuchat, 1995). In more recent watermelon outbreaks, *S. Oranienburg* and *S. Javiana* were the causative agents (Beuchat, 1995). Again, it is believed that *Salmonella* on the unwashed exterior of the fruit were introduced into the interior of the fruit by the act of cutting (Beuchat, 1995).

Salmonella spp. has also been associated with other types of produce. Other than melons, sprouts have been associated with *Salmonella* outbreaks. Seed sprouts present a special problem in that pathogens can be found in very low levels on the seeds, but can multiply to high levels during the sprouting process (FDA, 2001). In 2001, the California Department of Health found *S. Kottbus* in alfalfa sprouts that were produced by a single sprout producer. Outbreaks of *S. Kottbus* occurred in 4 states and caused acute diarrheal illness in 21 patients, and caused urinary tract infections in 3 patients (CDC, 2002). In 1988, an outbreak of *S. Saint-Paul* occurred in the United Kingdom (Beuchat, 1995). The outbreak involved 143 cases of infection and the vehicle food was raw bean sprouts (Beuchat, 1995). Also in 1988, bean sprouts infected with *S. Saint-Paul*, *S. Havana*, and *S. Muenchen* caused an outbreak in Sweden (Beuchat, 1995). In Bangkok, a survey of bean sprouts sold in four open markets was conducted (Beuchat, 1995). Of 344 samples, 30 (8.7%) were positive for *Salmonella* (Beuchat, 1995). The serotypes that were found were Lexington, Orion, Senftenburg, Tennessee, Poona, and Weltevreden. Some of the strains found were resistant to antibiotics (Beuchat, 1995).

Tomatoes have also been associated with *Salmonella* outbreaks. Studies have shown that *Salmonella* spp. are capable of growing in damaged, chopped, or sliced tomatoes (pH 4.1-4.5) held at 20-30°C (FDA, 2001). In 1990, an outbreak of *S. Javiana* in fresh tomatoes resulted in 176 cases of illness in Illinois, Michigan, Minnesota, and Wisconsin (Tauxe et al, 1997). In 1993, another outbreak occurred with tomatoes. The causative agent was *S. Montevideo*, which caused 100 cases of illness in the same states as the 1990 outbreak (Tauxe et al, 1997). Both outbreaks were traced back to a packer in South Carolina, who used a contaminated water bath to wash the tomatoes (Tauxe et al,

1997). The most recent multi-state outbreak of *Salmonella* in tomatoes occurred in 2000, with the causative agent being *S. Baildon* (FDA, 2001).

B. Microbiological Analysis of Raw Produce

1. Sample Selection and Preparation

Various methods can be utilized to examine the survival, growth, removal, or behavior of microorganisms in fresh produce. When conducting an experiment there are several important factors to consider. Factors can include type of produce, organisms being tested for, inoculum preparation, sample preparation, test method, and reporting results.

The type of fruit or vegetable, whether it be whole, cut, or if a certain part of the plant is used (i.e. stem, flower, leaf, or root), is an important factor to consider. When using whole or intact produce, problems can arise. Organic material or naturally occurring antimicrobials, produced by natural microflora or the produce itself, can cause inhibition or death of the microorganism being tested for (Beuchat et al, 2001). Blending or homogenizing of fruit pieces in diluent also presents problems. When plant tissues are blended or homogenized, antimicrobial compounds may be released, thus having a lethal effect on the microorganism being tested for (Beuchat et al, 2001).

2. Recovery of Indigenous and Inoculated Organisms

Prior to conducting the experiment, the organism or organisms being tested for and the inoculum preparation is an important factor to consider. At least 5 strains of the desired microorganism, either associated with foodborne disease outbreaks or isolated from produce or patients with illness associated from consumption, must be used (Beuchat et al, 2001). If a single strain is used, it must be tested against other strains for

resistance to the test conditions, otherwise a single strain that would be less tolerant to stress conditions would produce inaccurate results (Beuchat et al, 2001). In one study examining the effect of sanitizers on eliminating *Salmonella* from cantaloupe surfaces, Ukuku and Sapers used *Salmonella* Stanley H0558 (2001). If multiple strains are used, which is recommended, they must be examined for their ability to survive on the produce held under various environmental conditions or for their susceptibility to the test conditions (Beuchat et al, 2001). Also, strains must be examined for potential reactions against each other, such as production of inhibitory or lytic agents (Beuchat et al, 2001). Many studies have utilized the multiple strain mixtures. For example, Harris et al (2001) used five types of *Salmonella* to examine the efficacy and reproducibility of a produce wash in eliminating the bacteria on the surface of tomatoes. The strains of *Salmonella* that were used were Agona (alfalfa sprouts), Enteritidis (egg), Gaminara (orange juice), Montevideo (tomatoes), and Typhimurium (cattle feces) (Harris et al, 2001).

After pathogen selection, inoculum preparation is another important step. The strains should be cultured in a standard broth or agar medium and grown at temperatures representative of the strains' previous growth conditions (Beuchat et al, 2001). For example, if the strains were found in bovine feces, growth temperatures should be at 37°C, or if the strains were found on produce during storage, temperatures should be at either 5-12°C or 20-25°C (Beuchat et al, 2001). Strains should be transferred several times prior to inoculation of the produce. Although repeated transfers may reduce tolerance to environmental stresses, it is desirable to have cells with uniform cell type (Beuchat et al, 2001). Also during this phase, it may be desirable to have a marker. Markers such as nalidixic acid (50 µg/ml), rifampicin (80 µg/ml), or plasmids or proteins

with fluorescent capabilities can assist in bacterial recovery on non-selective or selective media, and inhibit growth of other microorganisms (Beuchat et al, 2001). If a marker is used, it is recommended that the marker be maintained for at least ten generations to assure the stability of the marker for studies involving multiplication of the bacteria on produce or for recovery methods involving a non-selective step (Beuchat et al, 2001).

After the strain(s) have grown and a marker is applied, inoculation of the whole or cut produce is the next step. Inoculation can be performed either by submerging the produce into a bacterial suspension, or by spot inoculating. Dip inoculation would be used in experiments where, in a commercial situation, the suspected point of contamination of the produce is during an immersion step (Beuchat et al, 2001). A disadvantage to dip inoculation is that the actual number of cells applied to the produce is not known and the acquired inoculum on each sample is highly variable (Beuchat et al, 2001). Some studies utilize the dip inoculation method. Ukuku and Fett (2002) inoculated cantaloupe surfaces with *Listeria monocytogenes* by submerging the whole fruit into a bacterial suspension containing 10^8 CFU/ml, agitating with a glove-covered hand for 10 minutes, and dried the produce in a biosafety cabinet for one hour. Ukuku and Sapers (2001) utilized a similar procedure for inoculating cantaloupe with *Salmonella* Stanley, by submerging the produce into 3 liters of inoculum, agitating with a glove-covered hand for ten minutes, and dried in a biosafety cabinet for one hour. Lukasik et al (2001) inoculated strawberries by immersing the samples for 2 minutes in one liter of deionized water, containing either *Salmonella* Montevideo, *Escherichia coli* O157:H7, various bacteriophages, or polio virus LSC1. An alternative to dip inoculation is spot inoculation, where a known cell density and volume of inoculum is applied to the

produce in small droplets. Spot inoculation simulates contamination that is caused by soil, workers' hands, or equipment surfaces (Beuchat et al, 2001). Lukasik et al (2001) inoculated tomatoes with bacterial or viral cultures by applying 25 µl of the cell suspension to the intact surfaces, stem scars, or surface scar areas. Harris et al (2001) used a micropipetter to apply 50 µl of 10^9 CFU/ml *Salmonella* suspension to the area around the blossom end of a tomato. Knudsen et al (2001) inoculated whole or cut strawberries with 15µl of either a mixed culture of *Salmonella* spp. or *Escherichia coli* O157:H7. Whether dip or spot inoculation is used, various factors must be considered. If the temperature of the produce is higher than that of the inoculum, internalization of the microorganisms may occur (Beuchat et al, 2001). Also, if produce with porous surfaces or areas are used, microorganisms may internalize, grow, or be protected from experimental treatments (Beuchat et al, 2001).

After inoculation, a wide variety of methods can be used to recover, detect, or enumerate the desired microorganisms. According to Beuchat et al (2001), the optimum protocol for recovering microorganisms is dependent on whether the surface, tissue, or both are being analyzed. To recover microorganisms from the surfaces or tissues, samples, whether whole or in portions, may be washed, blended, homogenized, stomached, macerated, or ground for direct plating or enrichment (Beuchat et al, 2001). For example, Burnett and Beuchat (2001) examined different methods of recovering *Salmonella* spp. from produce and herbs. Intact, inoculated produce samples were vigorously washed by hand in sterile stomacher bags with sterile 0.1% peptone water for 30 seconds. Products such as alfalfa sprouts, apple skins, lettuce leaves, and chopped tomatoes were placed into sterile stomacher bags with sterile 0.1% peptone water and

were stomached in a Stomacher 80 laboratory blender for 30 seconds. Another method used was to place samples in a 50-ml centrifuge tube with sterile 0.1% peptone water, and then the samples were homogenized in a rotor-stator homogenizer for 30 seconds at a medium speed setting. In another study examining the effect of sanitizer treatments on *Salmonella* Stanley attached to cantaloupe surfaces, Ukuku and Sapers (2001) immersed inoculated cantaloupe in either sterilized tap water, 1000 ppm chlorine, or 5% hydrogen peroxide. Cantaloupe were washed for 5 minutes by manually submerging and rotating to ensure complete coverage of the melon. Knudsen et al (2001) placed fresh or frozen strawberries, in groups of three, into stomacher bags with either 0.1% peptone water or 0.1 M phosphate buffer and samples were stomached for 90 seconds.

3. Effect of Ultrasound on Microorganisms

Ultrasound can be used for many types of applications in the food industry. For example, ultrasound can stimulate activity of cells or enzymes. Mason et al (1996) report that ultrasound can reduce the hatch time of fish eggs. At a frequency of 1 MHz for 35 minutes 3 times a day, fish eggs experienced a reduction in hatch time from 72 to 60 hours. In another study, when exposed to an ultrasonic frequency of 20 kHz, the enzyme α -chymotrypsin with casein as a substrate, experienced a two-fold increase in activity (Mason et al, 1996). One of the main uses of ultrasound is for cleaning and decontaminating surfaces, which is what this project will focus on.

Cleaning can be achieved by passing ultrasonic waves greater than 16 kHz through a fluid. When these waves pass through a liquid, alternating cycles of expansion and compression are produced (Raso et al, 1998). Small cavities or bubbles are created during the expansion phase. As these bubbles or cavities absorb energy, they grow in

size. When these bubbles or cavities can no longer absorb energy, they will violently collapse or implode. During implosion, there are two main effects. First, the collapse of the bubbles produces intense hydrodynamic shock waves, which can produce a scrubbing action that can dislodge soil and other materials from a surface (Scherba et al, 1991). Second, it is believed that high temperatures up to 5,500°C and pressures up to 50 mPa are attained, which can have a bactericidal effect (Raso et al, 1998). This entire process of cavity production and implosion is known as cavitation.

Earnshaw (1998) describes two types of cavitation: stable or transient. In stable cavitation, small bubbles are produced in a liquid. As ultrasonic waves pass through the liquid, the bubbles vibrate instead of growing and collapsing (Earnshaw, 1998). As the bubbles vibrate, they produce strong currents, which spread throughout the fluid. This effect is known as microstreaming, which provides a substantial force that rubs against cells and causes disruption of microbial cells (Leadley and Williams, 2002). In transient cavitation, ultrasonic energy passing through a liquid causes bubbles to rapidly form and collapse. As the bubbles collapse, the high temperatures and pressures created generate forces strong enough to remove matter from surfaces and can even damage the cell walls of microorganisms (Earnshaw, 1998).

The degree of ultrasonic cavitation can be affected by certain factors (Leadley and Williams, 2002). Frequency is an important part of cavitation. Lower frequencies (20 kHz) produce larger bubbles which, when they collapse, produce higher energies. Higher frequencies (2.5 MHz) have more difficulty producing bubbles, and cavitation might not occur. Temperature can also have an effect on cavitation. Higher temperatures lead to higher production of bubbles, but the intensity of their collapse is reduced because it is

believed that an increase in vapor pressure leads to a decrease in tensile strength. This can lead to decreased cavitation. The effect of higher temperature can be reduced if pressure is applied. Combining pressure (200 to 600 kPa) and temperature with ultrasound can increase the amplitude of ultrasonic waves, thus increasing the effectiveness of microbial inactivation.

Another proposed effect of ultrasound on microorganisms is the formation of free radicals. Sonication of water or liquid can lead to the formation of OH^\cdot and H^\cdot ions and hydrogen peroxide, which can have a bactericidal effect (Leadley and Williams, 2002). Production of these free radicals can damage DNA in bacterial cells. It is believed that hydroxyl radicals produced from sonication can break the hydrogen bonds in DNA and cause fragmentation (Leadley and Williams, 2002).

Many studies have been conducted examining the germicidal efficacy of ultrasound. Scherba et al (1991) examined ultrasound's ability to kill various microorganisms in an aqueous suspension. Cultures of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, feline herpesvirus type 1, and feline calicivirus, in aqueous suspensions, were exposed to 26 kHz ultrasound for 1, 2, 4, 8, 16, and 32 minutes. Bacterial numbers were reduced, possibly due to damage to the cytoplasmic membrane, with higher reductions at longer exposure times (Scherba et al, 1991). For the fungus, *T. mentagrophytes*, as exposure time increased, reduction increased (Scherba et al, 1991). For the viruses, viral infectivity was significantly reduced due to possible destruction of the viral envelope (Scherba et al, 1991)

Wrigley and Llorca (1992) examined ultrasound's ability to decrease *Salmonella* Typhimurium in skim milk, liquid whole egg, and brain heart infusion broth. Numbers of *S. Typhimurium* in brain heart infusion broth, after 0, 15, and 30 minutes of sonication, were reduced by more than 99% and viability of cells decreased as the temperature of sonication increased from 0°C to 40°C (Wrigley and Llorca, 1992). In skim milk, the log reduction of *S. Typhimurium* was not as dramatic as brain heart infusion broth, but a 2-log reduction was observed during ultrasonic treatment at 40°C (Wrigley and Llorca, 1992). Liquid whole egg appeared to protect the bacteria from sonication, with reductions being less than 1-log (Wrigley and Llorca, 1992).

Raso et al (1998) examined the lethal effect of ultrasonic waves (20 kHz), in combination with temperature and pressure, on *Yersinia enterocolitica*. A specially designed resistometer was used, where cell suspensions can be injected into a test chamber and be subjected to ultrasonic treatment with varying temperatures and pressures. Once the desired temperature and pressure had been achieved, 0.2 ml of the *Y. enterocolitica* suspension was injected into the test chamber, which contained citrate-phosphate buffer. After treatment, 0.1 ml samples were collected into tubes of sterile nutrient agar supplemented with 500 mg of Bacto-Dextrose. At ambient temperature and pressure, the lethal effect of ultrasonic waves was small, with the D-value of *Y. enterocolitica* being 1.5 minutes. Increasing pressure from 0 to 300 kPa reduced the D-value from 1.5 minutes to 0.28 minutes. Further increasing pressure from 300 to 600 kPa reduced the D-value from 0.28 minutes to 0.20 minutes. The use of increased pressure increased the lethality of ultrasonic waves. Also, between 50°C and 58°C, the lethality of heat can be increased when used in combination with ultrasonic waves and pressure.

Pagan et al (1999) used the same resistometer and procedure to examine bacterial resistance to ultrasonic waves under pressure at nonlethal (manosonication) and lethal (manothermosonication) temperatures. In this experiment, the bacterial strains that were used were *Streptococcus faecium*, *Listeria monocytogenes*, *Salmonella* Enteritidis, and *Aeromonas hydrophila*. The decimal reduction times for *S. faecium*, *L. monocytogenes*, *S. Enteritidis*, and *A. hydrophila* at 62°C were 7.1, 0.34, 0.024, and 0.0096 minutes, respectively. D-values obtained during manosonication treatment (40°C, 200 kPa, 117 μ m) were 4.0, 1.5, 0.86, and 0.90 minutes, respectively.

C. Surface Area Measurement of Raw Foods

Determination or estimation of the surface area of various types of food can have a wide variety of applications. Surface area is important when calculating transfer of heat, water vapor, gases, pesticides, and nutrients moving in and out of food products (Clayton et al, 1995). In terms of food microbiology, surface area determination of foods can help report counts of microorganisms per unit of surface area, rather than counts per milliliter of rinse volume.

Few techniques have been developed to determine surface area of irregularly shaped objects. It can be difficult to determine or estimate the surface area of fresh produce because of its irregular shape or exterior irregularities. However, several studies have developed methods to estimate the surface area of various types of foods.

Frechette and Zahradnik (1966) examined the relationship of surface area and weight for McIntosh apples. 75 apples, within the range of 2.25 to 3.25 inches in diameter, were weighed and cut into slices parallel to and through the core. The apple

slices were peeled so that enough flesh was on the peel to facilitate tracing onto graph paper. After drying, the paper peels were cut out and allowed to dry for several days in order to ensure constant weight. From the paper peels, one-inch paper squares were cut out and weighed. The weight of the one-inch squares gave the weight per square inch. Then, the total paper peel tracings from a given apple were weighed. The total weight of paper peel tracings from a given apple, divided by the weight per square inch, gave the surface area of the apple in square inches. After performing a linear and curvilinear regression, an equation was developed to determine surface area of McIntosh apples. The equation is $Y_L = 7.82 + 0.11W$, where Y_L is the surface area in square inches, and W is the weight in grams.

Thomas (1978) used a similar method to determine the surface area of chicken, duck, and poultry carcasses. Bird carcasses were chilled, weighed, and their overall length was measured. The skin of the carcass was carefully removed in order to avoid stretching or distortion. The skin was placed onto waxed paper and traced using a felt-tip pen. The tracings were traced again onto calibrated sheets of waxed paper, cut out, then weighed in order to determine external and total surface area. After performing linear regressions, the following equations were developed to estimate carcass surface area, where x is the weight of the eviscerated carcass in grams:

Chickens:

$$\text{External surface area (cm}^2\text{)} = 0.67x + 536$$

$$\text{Total surface area (cm}^2\text{)} = 0.87x + 635$$

Ducks:

$$\text{External surface area (cm}^2\text{)} = 0.66x + 583$$

$$\text{Total surface area (cm}^2\text{)} = 0.81x + 696$$

Turkeys less than 7 kg:

$$\text{External surface area (cm}^2\text{)} = 0.36x + 1219$$

$$\text{Total surface area (cm}^2\text{)} = 0.45x + 1293$$

Turkeys over 7 kg:

$$\text{External surface area (cm}^2\text{)} = 0.10x + 3025$$

$$\text{Total surface area (cm}^2\text{)} = 0.13x + 3480$$

Hershko et al (1998) examined the use of atomic force microscopy to determine the surface area of garlic and onion skins. Garlic and onion skins were peeled manually and were treated with chloroform 6 times to remove epicuticular wax from the skins. One hour after chloroform treatment, skins were studied by atomic force microscopy, using a Nanoscope II atomic force microscope. Image processing and Arc/Info software were used to interpret the data (Hershko et al, 1998).

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Running Head: Alternative Rinse Protocols for Recovery of *Salmonella* spp. from Produce Surfaces

Alternative Rinse Protocols for Recovery of *Salmonella* spp. from Produce Surfaces

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ABSTRACT

Fresh fruits and vegetables have been increasingly associated with outbreaks of food borne illness. Microorganisms on the surface of raw produce may be difficult to remove for decontamination or microbial sampling due to porous surfaces and microbial attachment. The objective of this study was to determine if ultrasonic treatment (40 kHz) of a rinse solution, with varying diluent temperatures and agitation times, can enhance removal and recovery of *Salmonella* spp. from produce surfaces. Strawberry, apple, and cantaloupe surfaces were spot inoculated with a 5-strain cocktail of nalidixic acid-resistant *Salmonella* spp. The produce (25°C) was immersed in 0.1% buffered peptone water in either a Whirl-Pak bag for manual shaking (60 seconds) or a sterile beaker for ultrasonic treatment (60 or 120 seconds). Additionally, diluent temperatures of either 25°C or 40°C were used with these sampling protocols. Diluents were spiral plated onto tryptic soy agar supplemented with 50 ppm of nalidixic acid. Ultrasonic treatment, with varying diluent temperatures and agitation times, did not enhance recovery of *Salmonella* spp. from produce surfaces. Counts of *Salmonella* spp. recovered from produce surfaces ranged from 2.2-2.7 log CFU/ml for strawberries, and 4.5-4.7 log CFU/ml for apples and cantaloupe, with no significant differences between treatments.

Fresh produce can harbor a wide variety of microorganisms, some of which can be pathogenic to humans. Since these products undergo a minimal amount of processing, the occurrence of some of these microorganisms is not uncommon. The surfaces of raw produce can serve as attachment sites for microorganisms, which can make removal of these microorganisms difficult (10). The surface can act as a barrier, but if the barrier is damaged, spoilage or pathogenic microorganisms can be introduced into the produce, thus making the product unfit for consumption.

Contamination of produce by microorganisms may occur during preharvest or postharvest practices. In preharvest operations, produce may come into contact with soil, water, animal feces, or improperly treated manure, which may harbor potentially pathogenic microorganisms. Soil can harbor a wide variety of microorganisms, including pathogens such as *Clostridium botulinum*, *C. perfringens*, *Listeria monocytogenes*, and *Bacillus cereus* (1). The closer the produce is to the ground, the higher the risk of contamination. Untreated water sources, which may be contaminated by raw sewage, can introduce pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, or *L. monocytogenes* into the produce. Improperly treated animal manure or human fecal matter, which is a significant source of pathogenic bacteria, may come into contact with produce and can harbor pathogens such as *Salmonella* spp., *E. coli* O157:H7, or *Cryptosporidium* (5).

Postharvest practices can be another source of contamination. Water, used for rinsing or cooling, may serve as a source of contamination if the water is reused or improperly treated (5). Improperly cleaned or sanitized equipment or utensils may introduce microorganisms onto or into the produce. The physical act of cutting or slicing

can introduce microorganisms, either on contaminated utensils or on the exterior of the product, into the interior of the produce. Also, workers handling the produce may introduce microorganisms into the produce, as well as processing water, equipment, or other workers, if they do not practice proper personal hygiene. Another source of postharvest contamination would be cross-contamination with other foods. Foods such as raw meat products have been known to harbor pathogenic bacteria such as *Salmonella* spp., *Campylobacter jejuni*, and *Escherichia coli* O157:H7.

Ultrasound has a wide variety of applications, one of which is cleaning. Ultrasonic cleaning relies on the concept of cavitation, where ultrasonic waves passing through a fluid solution produces small bubbles that expand and collapse (4). The collapse of these bubbles produces a force sufficient enough to damage cell wall structures or to remove particles from surfaces (4). Also, ultrasonic frequencies between 35-40 kHz can produce cavitation where shock waves and high heat are produced, which can injure cells (12). At the site of implosion, high temperatures (approximately 5,500°C) and high pressures (approximately 50 MPa) are momentarily produced (8). While many studies examined the germicidal efficacy of ultrasound, not many studies have been conducted evaluating the use of small exposures to ultrasonic energy to remove microorganisms from various surfaces.

The objective of this study was to determine if ultrasonic treatment (40 kHz) of a rinse solution, with differing diluent temperatures and agitation times, could enhance the removal and recovery of *Salmonella* spp. from different produce surfaces.

MATERIALS AND METHODS

Produce. Fresh, unblemished strawberries (California Giant) were purchased from a local retail outlet (Wal-Mart, Christiansburg, VA). Fresh, unblemished apples (Golden Delicious) and cantaloupe (Mission Variety) were purchased from a local food distributor (US Foodservice, Roanoke, VA).

Test strains and Media Preparation. Five serotypes of *Salmonella enterica* were used in this study. Serotypes Agona (alfalfa sprouts), Baildon (lettuce/tomato), and Michigan (cantaloupe) were obtained from Dr. Larry Beuchat of the University of Georgia. Serotypes Oranienburg (ATCC 9239) and Typhimurium (ATCC 14028) were obtained from the American Type Culture Collection (Manassas, VA). All strains were adapted to tolerate 50 µg/ml of nalidixic acid sodium salt MW=254.2 (ICN Biomedicals Inc., Aurora, OH). The growth medium used in this experiment was tryptic soy broth (Difco Laboratories, Sparks, MD) supplemented with 50 µg/ml of nalidixic acid (TSBN). The recovery medium used was tryptic soy agar (Difco) supplemented with 50 µg/ml of nalidixic acid (TSAN).

Inoculum Preparation. Prior to conducting the experiment, each strain was grown in TSBN for 24 hours at 35°C. At 24-hour intervals, each culture was transferred by single loop inoculation to new tubes of TSBN. To ensure that contamination had not occurred, cultures were periodically T-streaked onto Hektoen Enteric agar (Difco), and typical colonies (blue-green colonies with or without black centers) were inoculated to API 20E strips (bioMerieux, Hazelwood, MI) for identification. After at least 3 consecutive

transfers, all strains were mixed together in equal proportions to produce a five-strain cocktail containing approximately 1.0×10^8 CFU/ml.

Inoculation Procedure. All produce ($\sim 25^\circ\text{C}$) were inoculated and dried under a laminar flow hood. For strawberry inoculation, the cocktail was diluted in 0.1% buffered peptone water (Difco) dilution blanks to a level of approximately 1.0×10^6 CFU/ml. Each individual strawberry received 0.1 ml of the diluted cocktail to produce a final inoculum level of approximately 10^5 cells per strawberry. Strawberries were sampled in groups of three (considered one sample). For apples, 0.1 ml of the undiluted cocktail was applied to the apple to produce a final inoculum level of 10^7 cells per apple. For cantaloupe, 0.1 ml of the undiluted cocktail was applied to 3 different areas of the fruit to produce a final inoculum level of 10^7 cells per cantaloupe. Inoculated produce was dried on sterile metal racks under a laminar flow hood for 30 minutes.

Test Protocol. After drying, uninoculated or inoculated produce were placed into either a sterile Whirl-pak bag for manual agitation or sterile beakers for ultrasonic treatment. Various beaker sizes were used: 600 ml for strawberries, 800 ml for apples, and 4000 ml for cantaloupe. Set volumes of 0.1% buffered peptone water were used as diluent: 250 ml for strawberries, 300 ml for apples, and 1000 ml for cantaloupe. For ultrasonic treatment, diluent was applied to the beaker by pouring down the sides of the beaker to prevent washing off of the bacteria prior to treatment. Samples were treated using different agitation techniques (manual or ultrasound), diluent temperatures (25°C and 40°C) and exposure times (60 and 120 seconds). A Model 250D ultrasonic bath (40

kHz), purchased from VWR Scientific, was used for ultrasound treatments. Beakers were suspended in the ultrasonic bath by means of either a beaker holder or wire mesh basket, designed for this particular bath. Water levels in the bath were adjusted so that the bath water level matched the water level in the beaker. After treatment, diluent was plated in duplicate onto tryptic soy agar supplemented with 50 µg/ml of nalidixic acid, using a Model D Spiral Plater (Spiral Biotech, Inc., Bethesda, MD). Plates were incubated at 35°C for 48 hours. Triple sugar iron (Difco) and lysine iron agar slants (Difco) were used at random to confirm the presence of *Salmonella* spp. API 20E strips were used at random for final identification (bioMérieux).

Statistical Analysis. The experiment was a completely randomized design with subsamples. Three subsamples were taken for each treatment, and each treatment was replicated three times. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, NC) using Duncan's multiple range test to determine if there were significant differences ($\alpha = 0.05$) between treatments.

RESULTS & DISCUSSION

For all produce types, bacterial recovery was approximately 2 logs less than the starting inoculum level for all treatments. When plating samples of the uninoculated negative control, no nalidixic acid resistant background flora was found. Tables 1, 2, and 3 illustrate the counts of *Salmonella* spp. recovered from strawberry, apple, and cantaloupe surfaces. Based the statistical analyses, there were no significant differences in bacterial recovery between any of the treatments. It was hypothesized that increased diluent temperature and increased agitation time would help enhance bacterial recovery.

Based on the results, the effects of diluent temperature and agitation time did not enhance or diminish bacterial recovery.

The results from this experiment suggest that sonication did not enhance recovery of *Salmonella* spp. from produce surfaces. As seen in Appendices A and B, differences in counts of *Salmonella* spp. recovered by manual agitation or sonication were non-existent or relatively minor. Appendix C illustrates the levels of *Salmonella* spp. recovered from all three produce surfaces using four different sonication treatments. Again, differences in bacterial recovery between the treatments were minor.

There are a couple of theories on how ultrasonic waves may damage cells. When ultrasonic waves pass through a liquid, cavitation occurs where bubbles or cavities are produced, and eventually collapse (4). The collapse of these bubbles momentarily produces high pressures up to 100 Mpa and high temperatures up to 5000 K (4). The production of high pressures and temperatures bombard cell membranes and are strong enough to damage cell wall structures or remove particles from surfaces (4). Another theory suggests that ultrasonic waves passing through a liquid can form free radicals. When ultrasonic waves pass through water, H^+ , OH^- , and hydrogen peroxide, which are bactericidal, may be produced (6). The formation of these free radicals may attack the bacteria's DNA, thus breaking up the DNA into fragments (7). The phenomenon of cavitation or the formation of free radicals may have killed some of the *Salmonella* spp. during sonication of the samples.

If cellular death occurred during treatment, *Salmonella* spp. might have been susceptible to sonication. Gram-positive cells may be more resistant to ultrasonic cavitation than Gram-negative cells because Gram-positive cells have a thicker cell wall

and a more tightly adherent layer of peptidoglycan (4). It has also been suggested that rod shaped bacteria are more susceptible to ultrasound than coccus-shaped bacteria (4). However, in a study examining the germicidal efficacy of ultrasound on various Gram-positive and Gram-negative bacteria, Scherba et al (9) found that there was no significant difference in the percent of Gram-positive cells killed versus the percent of Gram-negative cells killed. Scherba et al (9) suggested that morphological features did not seem to be an important factor, but that ultrasonic waves may have damaged the inner cytoplasmic membrane of the cells, not the cell wall. Whether ultrasonic waves damage the cell wall or cytoplasmic membrane, the exact mechanism of the germicidal efficacy of ultrasound is still not known.

The surface topography of the produce may also have had an effect on bacterial recovery. For some types of produce, the surface can be complex and pathogens may tightly adhere or be absorbed into porous surfaces, thus making removal more difficult (10). Conditions where areas of the produce are exceptionally porous may promote or inhibit growth of the bacteria, as well as protect the cells from the treatment conditions (2). Also, if pathogens are present on the exterior of the produce, the physical act of cutting can introduce them into the interior of the product, where they can grow and proliferate.

For strawberries, inoculum was quickly absorbed into the fruit soon after inoculation. *Salmonella* spp. may have either been absorbed into the fruit or were protected from treatment conditions if the bacteria became lodged underneath the seeds found on the exterior of the fruit. Knudsen et al (6) reported that inoculum was absorbed into the strawberry, thus making it difficult to visually determine if the inoculum had

dried. If internalization of *Salmonella* spp. occurred on the strawberries, it may account for the higher variation between counts for each treatment, as compared to counts recovered from apples or cantaloupe.

For apples, the log counts of *Salmonella* spp. recovered appeared to be close to each other. Counts for *Salmonella* spp. recovered from apples were approximately 3 logs lower than the starting inoculum level. Unlike strawberries and cantaloupe, apple surfaces are not as porous. The surface of an apple is smooth, thus lacks areas where bacteria can be protected from treatment. Internalization of the bacteria is a possibility, but unlikely because for internalization to occur, a negative temperature differential, where the temperature of the produce is higher than the temperature of the inoculum, must occur (2). With few areas on the apple to shield bacteria from treatment, it is possible that some of the *Salmonella* spp. inoculated onto the apple surfaces may have died during drying. Burnett and Beuchat (3) reported that reductions in the number of *Salmonella* recovered from produce may be a result of the death of cells during drying.

For cantaloupe, the amounts of *Salmonella* spp. recovered were also close to each other. Much like apples and strawberries, counts of bacteria recovered were approximately 3 logs lower than that of the starting inoculum level. Because of the exceptionally porous surface on cantaloupe, some of the bacteria might have been shielded from treatment conditions. Studies have shown that *Salmonella* spp. are capable of surviving on cantaloupe surfaces for up to six days (11).

The results of this study suggest that ultrasonic treatment of a rinse solution did not enhance or diminish recovery of *Salmonella* spp. from produce surfaces. Also, the use of differing diluent temperatures and agitation times did not appear to enhance or

diminish bacterial recovery. If pathogenic bacteria are found on produce surfaces, damage to produce surfaces or the physical act of cutting can introduce these microorganisms into the interior of the product, where they can grow and proliferate. While ultrasound, along with differing diluent temperatures and agitation times, did not enhance recovery of *Salmonella* spp. from produce surfaces, this area should be studied more extensively. Further research in this area can include examining different ultrasonic frequencies (less or greater than 40 kHz), types of diluent, diluent temperatures, different produce types, different spoilage or pathogenic microorganisms, and different agitation/exposure times.

ACKNOWLEDGEMENTS

This research was partially funded through the Cooperative State Research, Education, and Extension Service of the U.S. Department of Agriculture, Special Research Grants Program - Food Safety (Project Number 99-34382-8463).

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Table 1. Recovery of *Salmonella* spp. per milliliter of diluent from strawberry^c surfaces using different agitation methods, diluent temperatures, and agitation times (n=9 for each treatment).

Agitation Method	Diluent Temperature (°C)	Agitation Time (sec)	Log CFU/ml \pm sd
Manual (Uninoculated)	25	60	ND ^b
Manual	25	60	2.77 ^a \pm 0.27
Manual	40	60	2.26 ^a \pm 0.42
Sonication	25	60	2.51 ^a \pm 0.41
Sonication	40	60	2.26 ^a \pm 0.48
Sonication	25	120	2.67 ^a \pm 0.48
Sonication	40	120	2.21 ^a \pm 0.50

^a Means with the same letter are not significantly different ($\alpha = 0.05$)

^b ND = not detected

^c Produce inoculated with 10^5 cells per strawberry

Table 2. Recovery of *Salmonella* spp. per milliliter of diluent from apple^c surfaces using different agitation methods, diluent temperatures, and agitation times (n=9 for each treatment).

Agitation Method	Diluent Temperature (°C)	Agitation Time (sec)	Log CFU/ml \pm sd
Manual (Uninoculated)	25	60	ND ^b
Manual	25	60	4.57 ^a \pm 0.11
Manual	40	60	4.61 ^a \pm 0.08
Sonication	25	60	4.55 ^a \pm 0.16
Sonication	40	60	4.74 ^a \pm 0.09
Sonication	25	120	4.65 ^a \pm 0.09
Sonication	40	120	4.68 ^a \pm 0.10

^a Means with the same letter are not significantly different ($\alpha = 0.05$)

^b ND = not detected

^c Produce inoculated with 10^7 cells per apple

Table 3. Recovery of *Salmonella* spp. per milliliter of diluent from cantaloupe^c surfaces using different agitation methods, diluent temperatures, and agitation times (n=9 for each treatment).

Agitation Method	Diluent Temperature (°C)	Agitation Time (sec)	Log CFU/ml \pm sd
Manual (Uninoculated)	25	60	ND ^b
Manual	25	60	4.70 ^a \pm 0.09
Manual	40	60	4.57 ^a \pm 0.29
Sonication	25	60	4.54 ^a \pm 0.17
Sonication	40	60	4.57 ^a \pm 0.32
Sonication	25	120	4.74 ^a \pm 0.10
Sonication	40	120	4.79 ^a \pm 0.08

^a Means with the same letter are not significantly different ($\alpha = 0.05$)

^b ND = not detected

^c Produce inoculated with 10^7 cells per cantaloupe

Surface Area Prediction of Raw Produce

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ABSTRACT

Surface area determination of various foods can have several important applications. Surface area measurements are necessary when calculating the amount of coating to be applied to a food, amounts of storage and shipping space, transfer of compounds in and out of foods, as well as application of pesticides and gases. Determination of surface area may also help estimate microbial populations on food surfaces. The objectives of this study were to 1) evaluate a new computer imaging system to determine produce surface areas, 2) determine if there are statistical relationships between produce weight and surface area, and 3) develop equations to predict surface area based on weight measurements. For apples (n=79, mean weight=171.1 grams, S.D. 6.0), the mean surface area was 185.8 cm² (S.D. 7.0). For cantaloupe (n=84, mean weight=923.2 grams, S.D. 116.2), the mean surface area was 517.1 cm² (S.D. 46.5). For strawberries (n=80, mean weight=17.7, S.D. 4.6), mean surface area was 37.2 cm² (S.D. 4.6). Using simple linear regression, it was found that there was a statistical relationship between weight and surface area. From the regression analyses, equations were developed to predict surface area from weight measurements.

INTRODUCTION

Determination or estimation of the surface area of various types of food can have a wide variety of applications. Turrell et al (1946) stated “Research studies in all fields of entomology, pathology, physiology, and chemistry often require estimates of surfaces and volumes of spherical fruits nuts and vegetables. Surface and volume measurements enter into calculations of amounts of space for storage and shipping, and of sprays, toxic gases, and dusts which are applied in control of pests; they are also needed in calculating respiration, water losses or absorption and temperature data.” Surface area is important when calculating the amount of coating to be applied to food products in order to extend shelf-life, as well as the transfer of heat, water vapor, gases, pesticides, and nutrients moving in and out of food products (Hershko et al, 1998, Clayton et al, 1995).

In terms of food microbiology, microorganisms are generally found on the surfaces of produce, rather than the interior. It can be difficult to determine surface area of produce due to differing shapes, thickness, and surface topography. Some processing technologies are designed to achieve a certain \log_{10} reduction of a specific microorganism based on weight or volume of the product, but this approach is not reasonable for produce because of the high variation between weight and surface area (Beuchat et al, 2001). A standard method must be developed in order to calculate or determine microbial populations on the surfaces of produce (Beuchat et al, 2001).

Several studies have developed methods to estimate the surface area of various types of foods. Frechette and Zahradnik (1966) determined surface area of McIntosh apples by tracing apple peels onto graph paper and weighing the strips of paper. They developed an equation ($A = 0.87 + 0.11W$, where A is the area in square inches, and W is

the weight in grams) to estimate surface area based on weight measurements. Thomas (1978) used a similar technique to develop equations to predict the surface area of chicken, duck, and turkey carcasses. Clayton et al (1995) used non-linear regression to estimate apple surface area based on weight and volume measurements. Hershko et al (1998) used atomic force microscopy to map and calculate the surface area of onion and garlic skins. Preliminary work conducted at Virginia Polytechnic Institute and State University involved measuring the surface area of apples and potatoes. Latex was applied to apple and potato surfaces. The latex was peeled off, and analyzed using the ImagePro Software program. Equations were developed to predict surface area from weight measurements. For apples, $\text{area (cm}^2\text{)} = 44.81 + (0.67 \times W)$, where W is the weight in grams. For potatoes, $\text{area (cm}^2\text{)} = 24.94 + (0.58 \times W)$.

It can be difficult to determine or estimate the surface area of fresh produce because of its irregular shape or exterior irregularities. So far, no method has been reported to rapidly, and non-destructively, measure the surface area of produce. The objectives of this research is to use a computer imaging system to determine produce surface areas, to determine if there are statistical relationships between weight and surface area, and to develop equations to predict surface area based on weight measurements.

MATERIALS AND METHODS

Measurement System. Figure 1 illustrates the surface area measurement system, which was developed by Dah-Jye Lee, of the Department of Electrical and Computer Engineering at Brigham Young University, in Provo, Utah (Lee et al, 2002a, 2002b).

The measurement system consisted of a light box, a stainless steel turntable with an attached encoder, and a digital camera. A light box was designed to project diffused light to provide backlight illumination on the test object. The test object blocked the incoming light, so that the object image would appear black, while the background light would appear white during imaging. Backlight illumination was used to acquire cross sections of the object, which provides better image quality and high contrast for image segmentation. Test objects are placed on a stainless steel turntable, and are held in a fixed position using an object holder, which can be mounted at the center of the turntable. The encoder, located at the base of the turntable, triggered the digital camera to take images every time the table was rotated six degrees. The measurement system acquired and stored multiple images of the object. Using the cross section images of the object, the measurement system used surface fitting and approximation of a 3-D wire frame model in order to calculate surface area.

Calibration and Adjustment. Prior to measuring samples, calibration of the measurement system was required. Calibration was performed by attaching a 3-inch aluminum cylinder to the turntable. When running the calibration program, the cylinder had to be aligned so that the center of the cylinder closely matched a centerline that appeared on the computer screen. After calibration, a sphere of known surface area, calculated using $A = 4\pi r^2$ (where r is the radius of the sphere), was measured using the fixture. Three surface area measurements of the sphere were collected, and averaged. To calculate the percent adjustment, the following equation was used:

$$\% \text{ adjustment} = (\text{Measured surface area} / \text{Known surface area}) \times 100$$

Once the percent adjustment was calculated, the value could be entered on the software user interface under “Percentage Adjustment”. Percent adjustment was performed until the measured surface area matched the known surface area.

Fruit Measurements. Three produce types were measured: strawberries (California Giant), apples (Golden Delicious), and cantaloupe (Mission Variety). All samples were obtained from a local food distributor (U.S. Foodservice, Roanoke, VA). Samples that were damaged or blemished were not used. Stems from apples were removed. Prior to surface area measurement, the weight of each individual fruit was recorded. For each type of produce, at least 80 samples were measured. Samples were placed on the object holder on the flywheel, perpendicular to the core of the fruit. The sample was rotated by hand. As the sample rotated, the imaging software collected images every 6° of rotation, collecting a total of 30 images when the sample was rotated a total of 180°. The software calculated the surface area (mm²) of the produce once 30 images were collected. Three surface area measurements were collected for each individual sample. The coefficient of variation ((S.D./μ) x 100) was calculated to evaluate the precision of the surface area measurements.

Predictive Equations. For each type of produce, a simple linear regression was performed to see if there was a statistical relationship between weight (g) and surface area (cm²). Analyses were performed using the JMP Statistical Discovery Software (SAS Institute Inc., Cary, NC). Based on the regression analyses, an equation was developed to predict surface area, based on weight measurements.

RESULTS AND DISCUSSION

Produce samples were analyzed using the surface area measurement system. Table 1 illustrates the mean weight (g), mean surface area (cm²), and coefficient of variation for all three produce types. The coefficient of variation (C.V.) represents the mean C.V. (3 measurements) for *n* samples. Average C.V. was between 1.0% and 1.3%, indicating high precision and repeatability. C.V. was less than 3.0% for 95% of all samples tested, and greater than 3.0% for 5 of 80 apples, 4 of 84 cantaloupe, and 4 of 80 strawberries.

Figures 2, 3, and 4 are the linear regressions of produce weight and surface area. When examining the regression plots, surface area and weight data of strawberries appeared to fall within the 95% confidence interval, while data for apples had less points falling within the 95% confidence interval. When looking at individual strawberry weight, the largest strawberry was approximately 3 times the weight of the smallest strawberry. However, for apples, the largest apple was approximately 1.2 times the weight of the smallest apple. For cantaloupe, the largest cantaloupe was approximately 1.7 times the weight of the smallest cantaloupe. A possible reason why the regression line for apples appeared to be closer to zero than the other produce types is because there was greater variation in size for strawberries and cantaloupe. Greater variation in produce size may have produced a regression line that had a slope closer to 1.

From the regression analyses, the following equations were generated to predict surface area from weight measurements:

$$\text{Strawberry: Surface area (cm}^2\text{)} = 9.36 + [1.575 \times \text{weight (g)}]$$

$$\text{Apple: Surface area (cm}^2\text{)} = 49.56 + [0.766 \times \text{weight (g)}]$$

$$\text{Cantaloupe: Surface area (cm}^2\text{)} = 197.01 + [0.377 \times \text{weight (g)}]$$

From these equations, weight measurements can be used to calculate predicted surface area.

For strawberries, the R^2 value was 0.96, which indicates the model explained 96% of the variation. For apples, the R^2 value was 0.47, which indicates the model only explained 47% of the variation. For cantaloupe, the R^2 value was 0.75, which indicates the model explained 75% of the variation. Weight was significant in explaining surface area for all three types of produce ($P < 0.0001$).

From this study, the surface area measurement system can be used to determine the surface area of various types of foods and objects. From these measurements, predictive equations can be developed to calculate surface area based on weight measurements. However, these equations need to be validated with surface area and weight measurements of additional samples. Further research can include validation of the predictive equations, comparing measurements of the surface area measurement system to other methods, using more irregularly shaped produce such as cucumbers, or using different produce types in order to create more predictive equations of surface area based on weight.

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Table 1. Produce surface area and weight measurements.

Fruit	n	Mean Surface Area \pm S.D. (cm²)	* Mean C.V. (%) of Surface Area	Mean Weight \pm S.D. (g)
apple	79	185.8 \pm 7.0	1.3	171.1 \pm 6.0
cantaloupe	84	517.1 \pm 46.5	1.1	923.2 \pm 116.2
strawberry	80	37.2 \pm 7.2	1.0	17.7 \pm 4.6

* Coefficient of variation (C.V.) % represents the mean C.V. of averaged (3 measurements) C.V. for n samples.

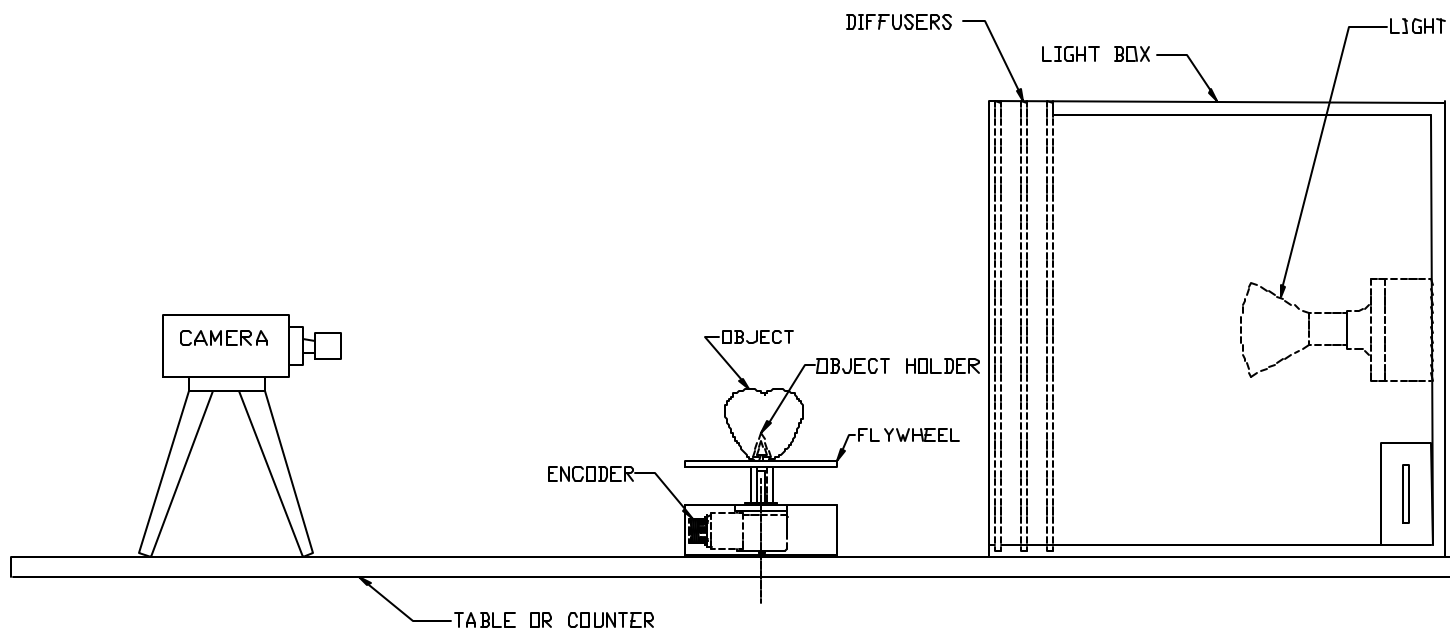
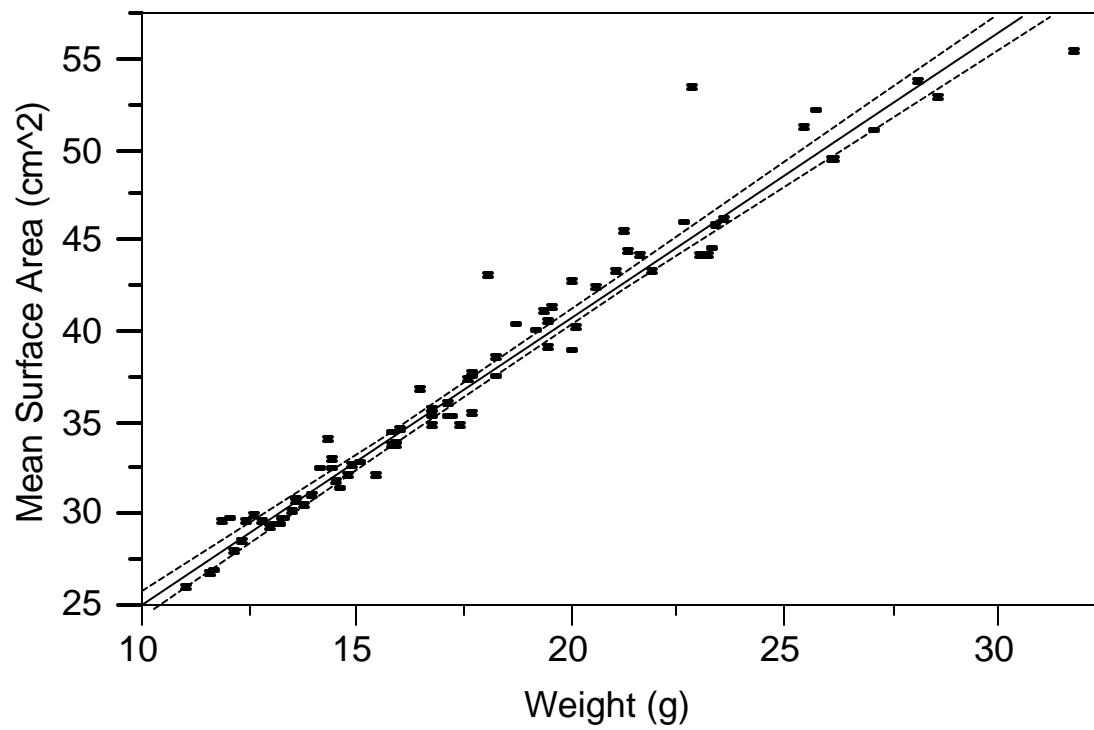


Figure 1. Diagram of surface area measurement fixture.



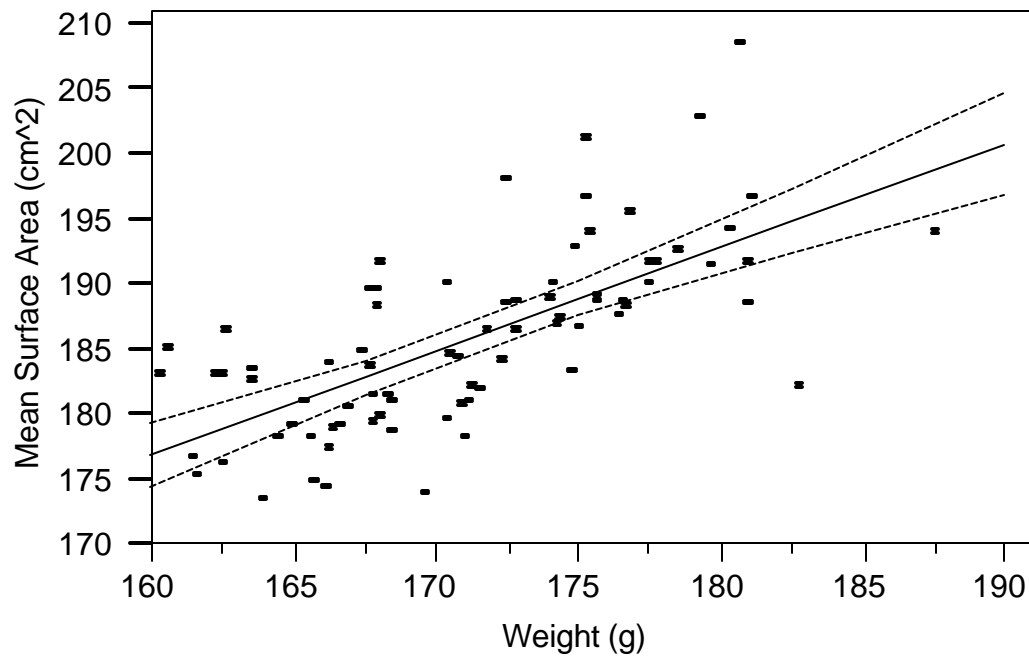


Figure 3. Regression plot of mean surface area (cm²) versus weight (g) of apples. Broken lines represent the 95% confidence interval.

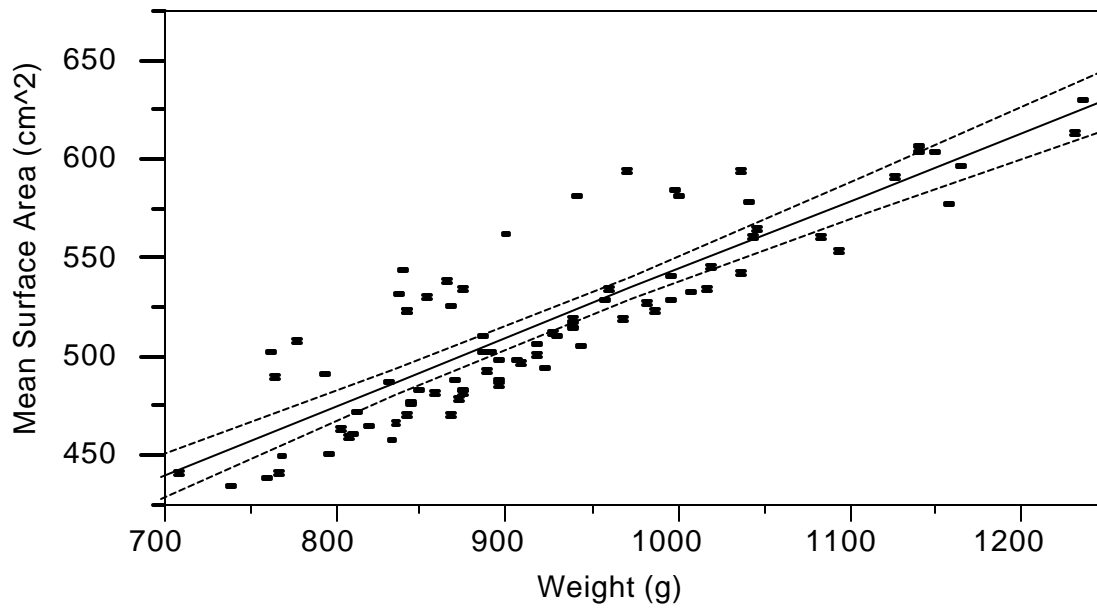


Figure 4. Regression plot of mean surface area (cm²) versus weight (g) of cantaloupe. Broken lines represent the 95% confidence interval.

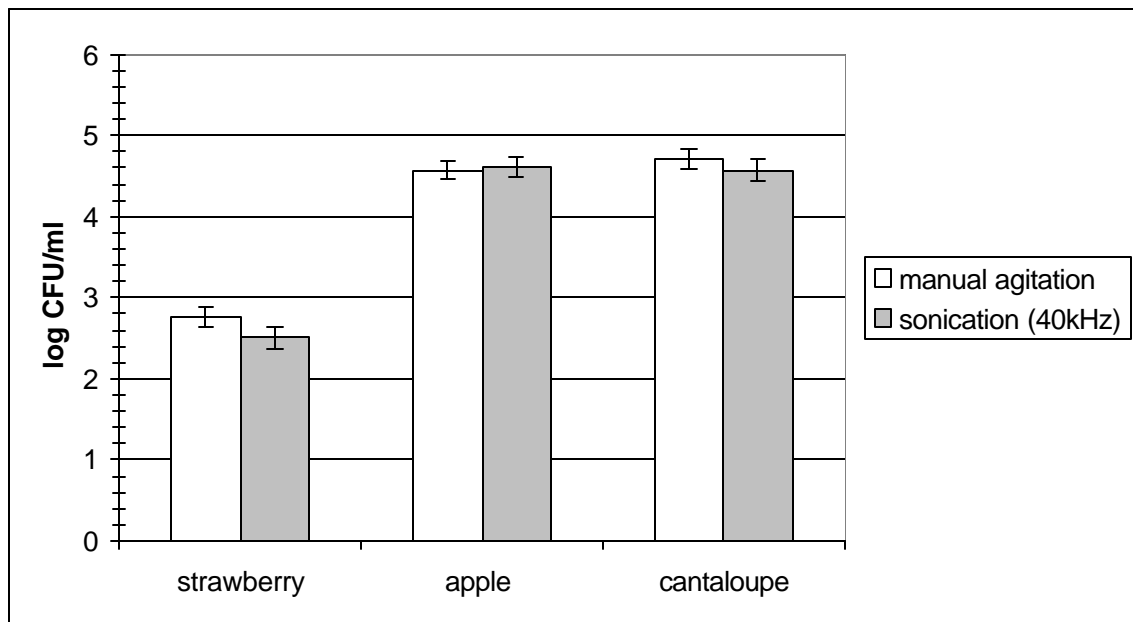
SUMMARY

Ultrasonic treatment of a rinse solution did not appear to enhance or diminish recovery of *Salmonella* spp. from produce surfaces. The effect of diluent temperature and agitation time did not appear to be significant. However, the use of ultrasound for either enhancing recovery or killing microorganisms should be further studied. Further research of this area can include using different ultrasonic frequencies (20 kHz or higher), alternate types of diluent or rinse agents, different diluent temperatures (20-50 °C), different produce types associated with foodborne outbreaks, other spoilage or pathogenic microorganisms, and longer or shorter exposure/agitation times.

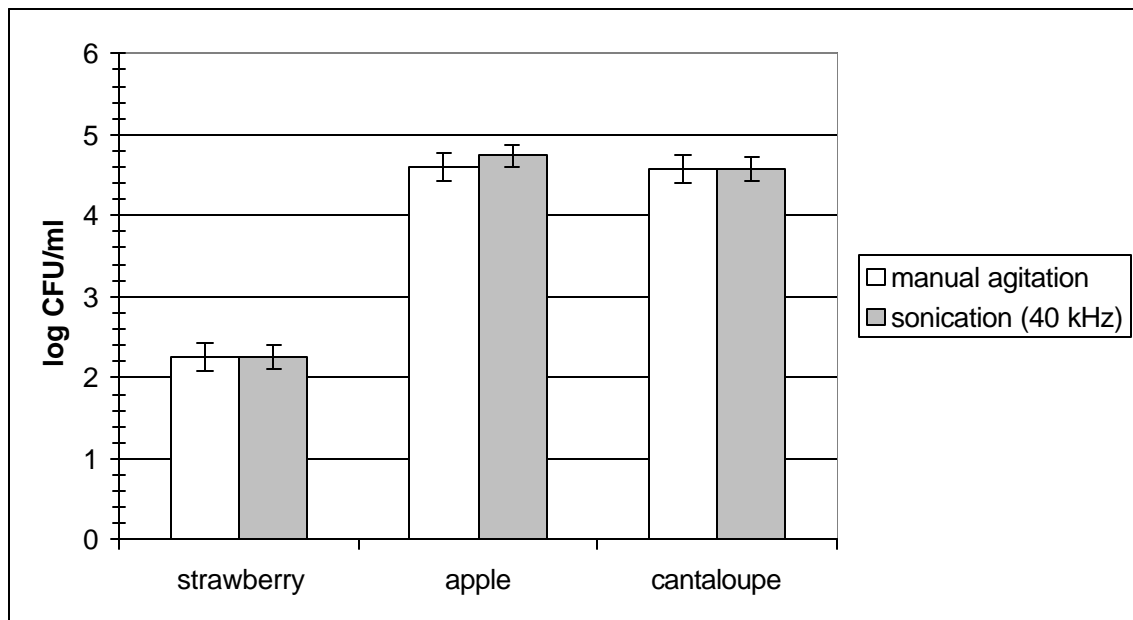
The surface area measurement system appears to provide a rapid method to determine the surface area of irregularly shaped objects or foods. Using data from the measurement system, equations can be developed in order to predict surface area based on weight measurements. Determination of the surface area of foods can help a food scientist with a wide variety of applications, and hopefully, apply this information to more accurately determine microbial populations on food surfaces.

APPENDICES

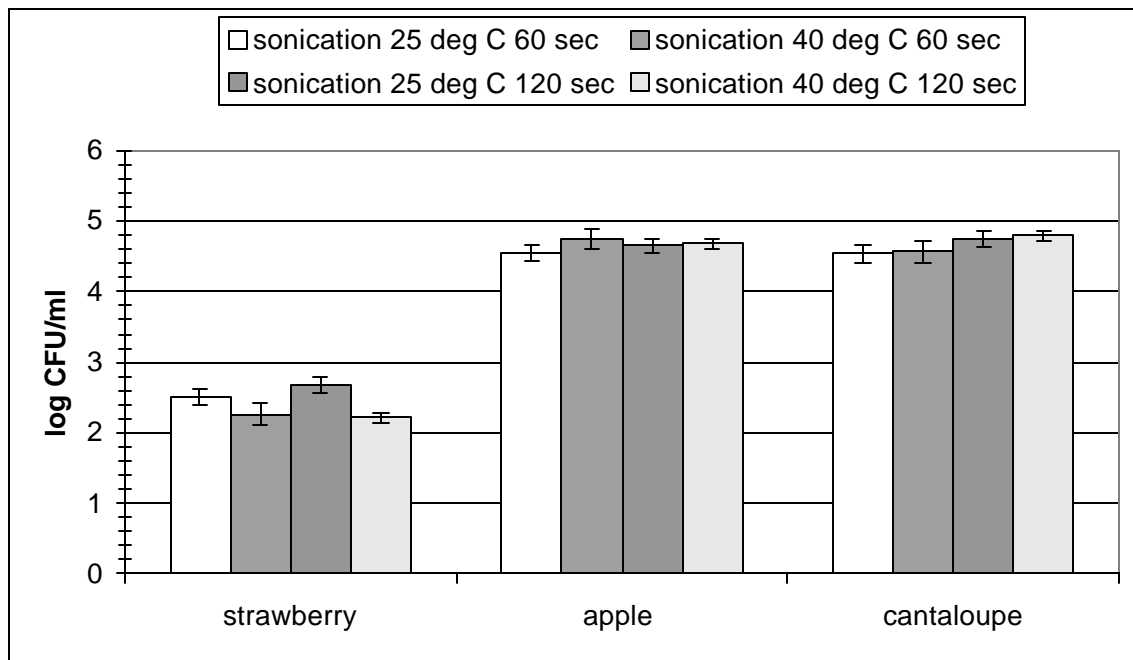
Appendix A. Recovery of *Salmonella* spp. from produce surfaces using manual agitation or sonication (40 kHz) for 60 seconds in 0.1% buffered peptone water (25° C).



Appendix B. Recovery of *Salmonella* spp. from produce surfaces using manual agitation or sonication (40 kHz) for 60 seconds in 0.1% buffered peptone water (40° C).



Appendix C. Comparison of various ultrasonic treatments utilizing different diluent temperatures (25°C and 40°C) and exposure times (60 and 120 seconds) to recover *Salmonella* spp. from produce surfaces.



VITAE

Gabriel Sanglay was born in Arlington, Virginia. After spending several years there, he and his family relocated to Herndon, Virginia where he graduated from Herndon High School in 1996. During the summer of 1999, he worked as a laboratory assistant for the Food Science and Technology Department at Virginia Polytechnic Institute and State University. In May of 2000, he attained his Bachelor's degree in Food Science and Technology. Soon after, he began his Master's program in Food Science and Technology in August of 2000. During his stay at Virginia Tech, he was a member of the Phi Sigma Biological Honor Society, the Fraternity of Phi Gamma Delta, the Food Science Club, the Institute of Food Technologists, and the International Association for Food Protection.